Effect of Increased Ascorbic Acid Blood Levels on the Ascorbic Acid Level in Treated and Non-treated Gingiva

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There is general agreement regarding the effects of ascorbic acid deficiency on the gingiva and periodontal tissues of experimental animals. Opinions have differed, however, regarding the significance of ascorbic acid deficiency in the etiology of gingival and periodontal disease in humans.[1-12] We were interested in determining whether the ascorbic acid level of the gingiva would be affected when the blood level was elevated by the systemic administration of ascorbic acid. An experiment was undertaken in humans for this purpose.

Materials and Methods

Forty-one male and female patients from twenty-five to sixty-six years of age with varying degrees of clinically detectable gingivitis served as experimental subjects. The patients were divided into 4 groups as follows:

GROUP 1—NON-SUPPLEMENTED, NON-TREATED GROUP (10 PATIENTS).—The diet was not supplemented, and the patients received no periodontal treatment. Ascorbic acid levels of the blood and the gingiva were determined at the outset of the experiment and repeated after the 2-week interval. This group provided an indication of the values of blood and gingival levels and of the fluctuations that occurred in the 14-day experimental period.

GROUP 2—SUPPLEMENTED, NON-TREATED (15 PATIENTS).—Ascorbic acid levels of the blood and gingiva were determined at the outset of the experimental period. The diets were supplemented by a daily dose of 250 mg. of ascorbic acid for 14 days, at the end of which time ascorbic acid level determinations in the blood and gingiva were repeated. The purpose of this group was to determine whether daily supplements of 250 mg. of ascorbic acid would significantly affect the blood level and, secondly, whether this change in the ascorbic acid level of the blood would be reflected in the ascorbic acid level of non-treated gingiva.

GROUP 3—SUPPLEMENTED, TREATED (6 PATIENTS).—Ascorbic acid level determinations were made of the blood and gingiva at the outset of the experimental period. The diets were supplemented by a daily dose of 250 mg. of ascorbic acid for the period of 14 days. The gingiva in one quadrant was treated by scaling and curettage, and the patient was instructed in oral physiotherapy. After 14 days the ascorbic acid levels of the blood and the gingiva on the treated side were determined.

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GROUP 4—NON-SUPPLEMENTED, TREATED (10 PATIENTS).—Ascorbic acid level determinations were done of the blood and gingiva at the outset of the experimental period. The diet was not supplemented with ascorbic acid. The gingiva in one quadrant was treated by scaling and curettage, and the patients were instructed in oral physiotherapy. After 14 days, ascorbic acid level determinations were done in the blood and gingiva. The purpose of this group was to determine whether the ascorbic acid level of the gingiva would be affected by periodontal treatment that consisted of scaling and curettage.

In all subjects, gingival biopsies were taken at the outset of the experimental period and repeated from a nearby site in the same quadrant after 14 days. Each biopsy consisted of marginal and contiguous attached gingiva.

The following method was employed for determining the ascorbic acid level in the blood and gingiva: Blood samples were taken from the finger tip with a 50-cu. mm. microconstriction pipette and transferred into 200 cu. mm. of 5 per cent trichloracetic acid in a microcentrifuge tube. The tube was capped and the contents thoroughly mixed. The procedure of Lowry, Lopez, and Bessey,[13] using larger samples, was followed for determining the ascorbic acid. Precision was checked by carrying out determinations in triplicate on random specimens.

The gingival specimens were prepared as follows: After the specimen was removed, it was washed free of surface blood, dried with filter paper, and weighed. Its volume was determined by measuring the amount of water it displaced. The tissue was immediately frozen in liquid nitrogen for a period of approximately 30 seconds. A steel mortar and pestle precooled in dry ice and 70 per cent alcohol were used to macerate the frozen specimen. The macerated gingiva was placed in 4 times its volume of ice-cold 5 per cent trichloracetic acid in an ice-cooled ground-glass homogenizer and ground until it was distributed throughout the solution. The ascorbic acid level was determined from samples of this mixture, using the procedures employed for the blood.

No attempt was made to alter the patient's usual dietary habits in the course of the experimental period other than providing the ascorbic acid tablets for the supplemented group.

Results

All patients presented clinically detectable inflammation with evidence of pocket formation. The gingival biopsies revealed varying degrees of chronic marginal inflammation, as evidenced by leukocytic infiltration, edema, and proliferative and degenerative changes in the connective tissue and epithelium (Fig. 1). In the quadrants treated by scaling and curettage (Groups 3 and 4) the clinical appearance of the gingiva was improved at the end of the 14-day period, and microscopic examination of the gingival biopsies revealed a reduction in the severity of inflammation, but not its complete elimination. No changes were observed in the clinical and microscopic features of the non-treated gingiva in the supplemented and non-supplemented groups.

GROUP 1—NON-SUPPLEMENTED, NON-TREATED.—At the outset of the experiment the ascorbic acid level of the blood in the patients in this group averaged 0.81 mg.

per cent, with a range of from 0.51 to 1.61. The ascorbic acid level of the gingiva averaged 1.49 mg. per cent, with a range of from 1.30 to 2.03 (Fig. 2). Except in one patient, the level of the gingiva was consistently higher than that of the blood. At the end of the experimental period the average blood level was 0.87 mg. per cent, with a range of from 0.58 to 1.51. The ascorbic acid level of the gingiva averaged 1.60 mg. per cent, with a range of from 0.61 to 2.30. There was some change in the ascorbic acid levels in the blood and gingiva but these were not statistically significant when the *t*-test was applied (Fig. 3). The ascorbic acid level of the gingiva was generally higher than the blood.

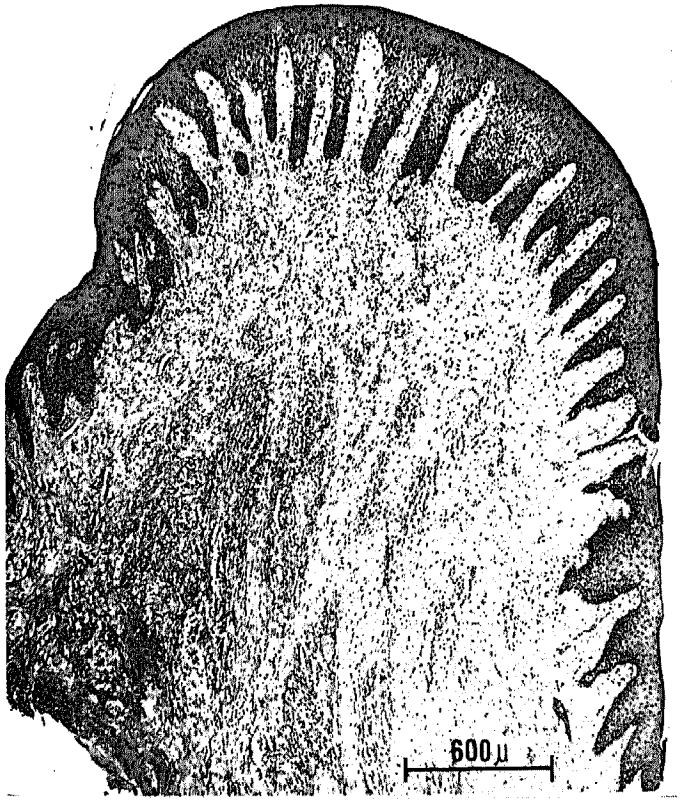

FIG. 1.—Representative biopsy of the inflamed marginal gingiva at the outset of the experiment, showing leukocytic infiltration and degeneration of connective tissue and epithelium. (Stain H & E.)

GROUP 2—SUPPLEMENTED, NON-TREATED.—At the end of the 2-week period the average ascorbic acid levels of the blood increased significantly from 1.0 mg. per cent, with a range of from 0.44 to 1.37, to an average value of 1.30 mg. per cent, with a range of from 0.97 to 1.80. The increase in gingival ascorbic acid level was not statistically significant. The initial average value was 1.54, with a range of from 0.87 to 2.63, and the final average value was 1.80 mg. per cent, with a range of from 1.10 to 3.56 (Figs. 4 and 5).

GROUP 3—SUPPLEMENTED, TREATED.—At the end of the 2-week period there was a

significant increase in the average blood level value from 0.91 mg. per cent, with a range of from 0.54 to 1.29, to 1.34, with a range of from 0.97 to 2.00. There was no significant change in the ascorbic acid value of the gingiva. The average value at the outset was 1.96 mg. per cent, with a range of from 1.30 to 3.28. The final average value was 1.97 mg. per cent, with a range of from 1.07 to 3.49 (Figs. 6 and 7).

GROUP 4—NON-SUPPLEMENTED, TREATED.—In this group there were no significant changes in the ascorbic acid levels of the blood or treated gingiva at the end of the

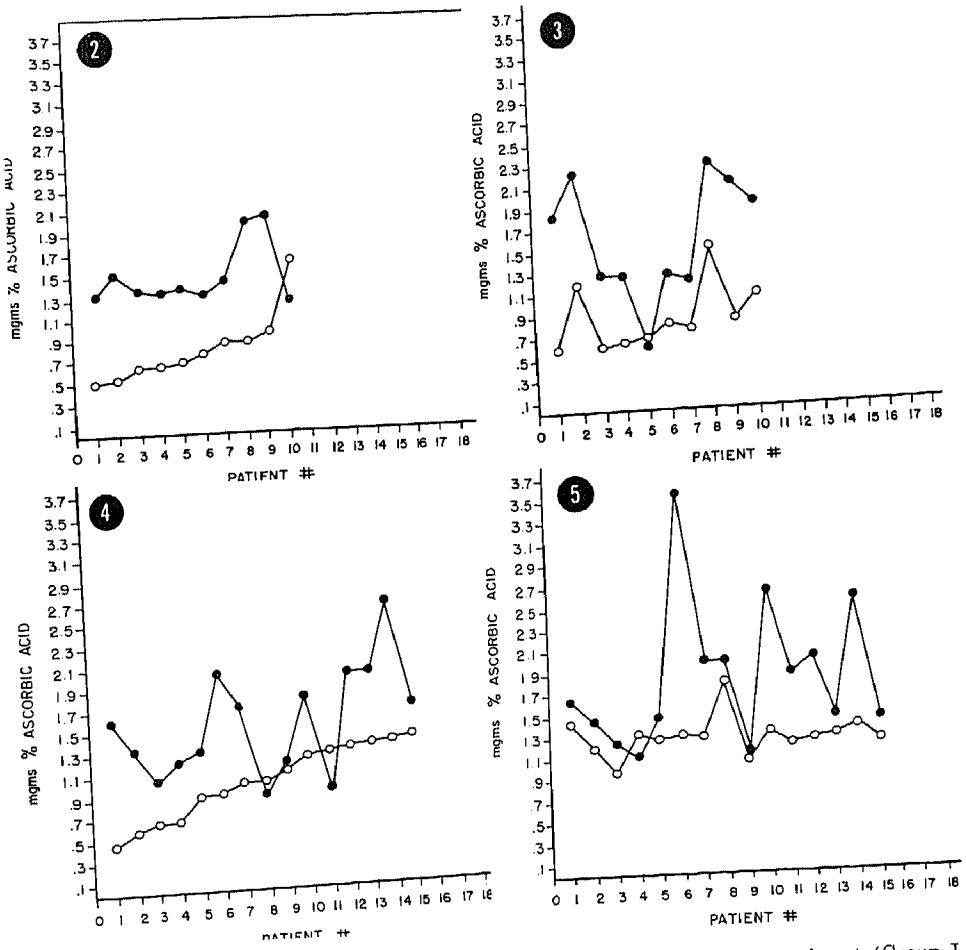

FIG. 2.—Ascorbic acid levels in the blood and gingiva at the outset of the experiment (Group I—non-supplemented, non-treated). *White dots* = blood; *black dots* = gingiva. The patients are arranged in ascending order according to the initial blood levels.

FIG. 3.—Ascorbic acid levels in the blood and gingiva after 14 days (Group I—non-supplemented, non-treated). The changes were not significantly different from those at the outset of the experiment (compare with Fig. 2). *White dots* = blood; *black dots* = gingiva.

FIG. 4.—Ascorbic acid levels in the blood and gingiva at the outset of the experiment (Group II—supplemented, non-treated). *White dots* = blood; *black dots* = gingiva.

FIG. 5.—Ascorbic acid levels in the blood and gingiva after 14 days (Group II—supplemented, non-treated). The blood levels were significantly increased. The gingival increase was not statistically significant (compare with Fig. 4). *White dots* = blood; *black dots* = gingiva.

experiment. The initial values in the blood were an average of 0.81 mg. per cent, with a range of from 0.43 to 1.20. The final average value in the blood was 0.95, with a range of from 0.32 to 1.60. In the gingiva, the initial average value was 1.63 mg. per cent, with a range of from 0.99 to 2.71, and the final average value was from 1.80 mg. per cent, with a range of from 1.09 to 2.80. The treatment of the gingiva did not produce an alteration in the ascorbic acid level (Figs. 8 and 9).

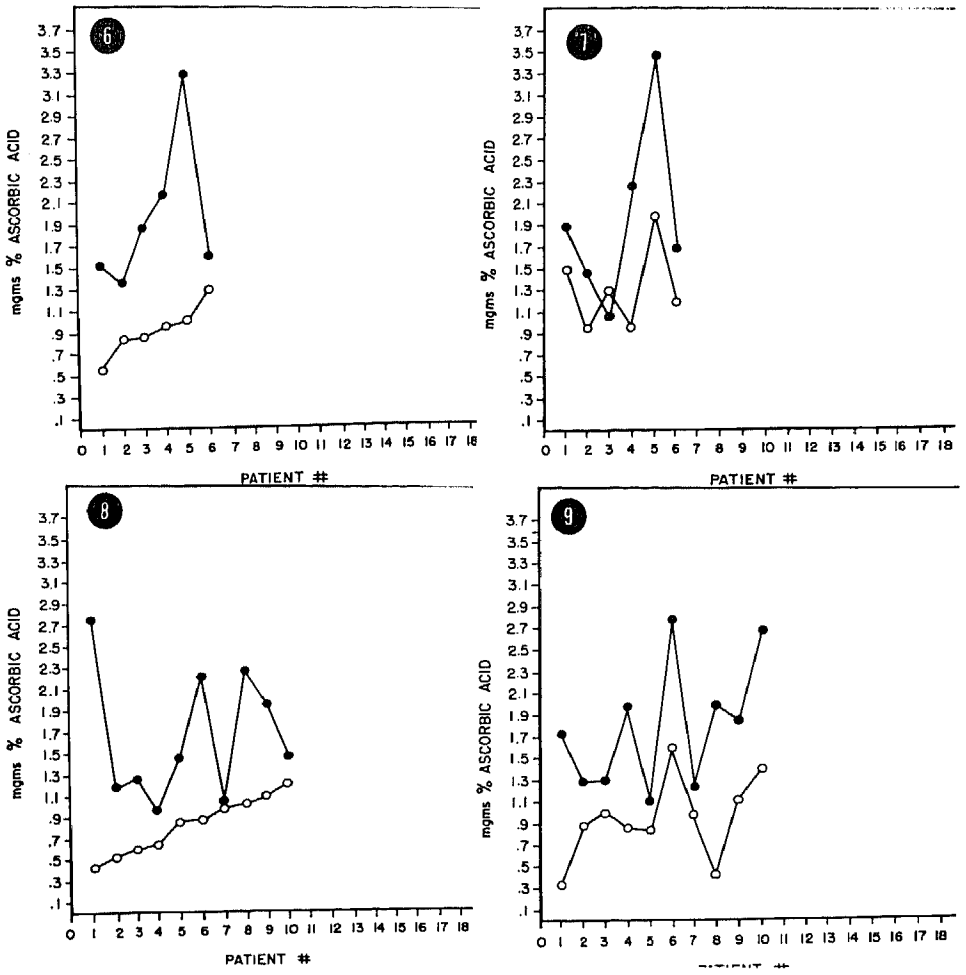

FIG. 6.—Ascorbic acid levels in the blood and gingiva at the outset of experiment (Group III—supplemented, treated). *White dots* = blood; *black dots* = gingiva.

FIG. 7.—Ascorbic acid levels in the blood and gingiva after 14 days (Group III—supplemented, treated). The blood levels were significantly increased. The levels in the treated gingiva were not (compare with Fig. 6). *White dots* = blood; *black dots* = gingiva.

FIG. 8.—Ascorbic acid levels in the blood and gingiva at the outset of the experiment (Group IV—non-supplemented, treated). *White dots* = blood; *black dots* = gingiva.

FIG. 9.—Ascorbic acid levels in the blood and gingiva were not significantly altered after 14 days (Group IV—non-supplemented, treated) (compare with Fig. 8). *White dots* = blood; *black dots* = gingiva.

Discussion

Our primary interest was in determining whether the ascorbic acid level of the gingiva would be affected by increasing the ascorbic acid level in the blood. The findings indicated statistically significant increases in the ascorbic acid level in the blood without comparable increases in the gingiva. The ascorbic acid blood levels at the beginning of the experiment were within the generally accepted normal range.[11-16] Perhaps the effect on the ascorbic acid level in the gingiva would have been greater if the ascorbic acid blood levels had been below normal at the outset of the experiment. It is interesting, however, to note the existence of normal ascorbic acid blood levels in a random selection of patients with varying degrees of clinically detectable gingival disease.

With few exceptions, the ascorbic acid levels were higher in the gingiva than in the blood before and after the ascorbic acid supplements. Because all patients presented some degree of clinically detectable gingival inflammation throughout the experiment, we are unable to comment regarding the ascorbic acid level of normal gingiva or its relationship to the blood level. It will be difficult to obtain the latter information because even in clinically normal gingiva there is some degree of microscopic inflammation at the base of the sulcus.

In addition to the clinical changes, the gingiva in all patients presented edema and leukocytic infiltration in the connective tissue, with proliferative and degenerative changes in the epithelium. The severity of the inflammatory changes was not related to the ascorbic acid levels in the blood or gingiva. Such a relationship might have existed in patients with subnormal ascorbic acid levels. It would be necessary to evolve a method of calibrating the inflammatory reaction to local irritants of different severity and duration, in order to establish whether the gingival response is affected by the ascorbic acid level.

Treatment of the gingiva by removing the irritants did not appear to influence the ascorbic acid levels of the gingiva in supplemented or non-supplemented patients; nor did the clinical or microscopic features of the treated gingiva appear to be affected by the supplements.

The dosage of the ascorbic acid supplements and the duration of the experimental period were sufficient to elevate the ascorbic acid levels in the blood. The possibility exists that, had the dosage been greater and the experimental period longer, the ascorbic acid levels of the gingiva would also have been significantly increased. However, the findings suggest that this would not happen in patients with normal ascorbic acid blood levels.

No attempt was made to control the patients' diets during the experimental period other than that certain subjects were given supplements of ascorbic acid. We were interested in the effect of the blood level on the ascorbic acid level of the gingiva, regardless of whether the blood level was elevated by diet, supplementation, or both.

Summary

In a random selection of patients with different degrees of gingival inflammation the ascorbic acid levels in the blood were within normal range.

Elevating normal ascorbic acid blood levels by daily dietary supplements of 250 mg.

of ascorbic acid did not significantly affect the ascorbic acid levels in treated and non-treated gingiva.

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